The Relationship Between Osteoprotegerin/RANKL Axis and Arterial Stiffness in Osteopenic/Osteoporotic Renal Transplantation Recipients

Böbrek Nakilli Hastalarda Osteoprotegerin ile Arteryal Damar Sertliği ve Osteoporoz Arasındaki İlişki

ABSTRACT

OBJECTIVE: Cardiovascular diseases are the main reason of death in patients with renal transplantation (Rtx). Osteoprotegerin (OPG) is produced by osteoblasts and is linked to increased cardiovascular risk in Rtx. OPG acts as a decoy receptor binding receptor activator of nuclear factor kappa-B ligand (RANKL) and this interaction plays a role in bone resorption and vascular function. This study aimed to investigate the relation between OPG, RANKL, osteoporosis and arterial stiffness in Rtx patients.

MATERIAL and METHODS: This cross-sectional study included 80 adult Rtx recipients. Femoral neck mineral density was obtained by dual-energy X-ray absorptiometry. Serum OPG and RANKL were measured by the ELISA method. Pulse-wave analysis was measured in the carotid and femoral arteries using a pulse wave velocity (PWV) machine.

RESULTS: Patients were divided into two groups as normal (n:24) and osteopenia/osteoporosis group (n:56). Body mass index was significantly lower in the osteopenic/osteoporotic group compared to the normal group. Pulse wave velocity was positively correlated with age (r:0.204,p:0.072), osteoprotegerin (r:0.219,p:0.052), calcium x phosphate product (r:0.605,p:<0.001), and systolic blood pressure (r:0.198,p:0.058) and negatively correlated with RANKL (r:-0.261,p:0.020) and creatinine clearance (r:-0.220,p:0.051). PWV was independently predicted by calcium x phosphate product but not creatinine clearance, RANKL, osteoprotegerin and systolic blood pressure.

CONCLUSION: In our study, serum calcium x phosphate product but not OPG and RANKL levels were found to be the main predictor of arterial stiffness in Rtx patients.

KEY WORDS: Renal transplantation, Osteoporosis, Osteoprotegerin, RANKL, Pulse wave velocity

ÖZ

AMAÇ: Kardiyovasküler hastalıklar böbrek transplantasyonu (Btx) olan hastalarda mortalitenin ana nedenleri arasındadır. Osteoprotegerin (OPG), osteoblastlar tarafından üretilir ve BTx'li hastalarda artmış kardiyovasküler risk ile bağlantılıdır. Normal popülasyonda OPG, serumda NF-κB ligandının (RANKL) bir reseptörü olarak davranır ve bu etkileşimin kemik erimesi ve vasküler fonksiyonlar üzerinde önemli bir rol oynadığı tespit edilmiştir. Literatürde Btx'li hastalarda bu etkileşimle ilgili yeterli bilgi bulunmamaktadır. Bu nedenle, çalışmamızda Btx'li hastalarda OPG, RANKL, osteoporoz ve arteriyel sertlik arasındaki ilişki araştırılmıştır.

GEREÇ ve YÖNTEMLER: Bu kesitsel çalışmaya 80 erişkin Btx'li hasta dahil edilmiştir. Femur boynu mineral yoğunluğu dual-enerji X-ışını soğurma (DEXA) yöntemiyle elde edildi. Serum OPG ve RANKL ELISA yöntemi ile ölçüldü. Nabız dalga analizi karotid ve nabız dalga hızı (NDH) makinesi kullanılarak femoral arterlerden ölçüldü.

BULGULAR: Hastalar osteopeni/osteoporoz grubu (n:56) ve normal (n: 24) olarak iki gruba ayrıldı. Vücut kitle indeksi normal gruba göre osteopenik/osteoporotik grupta anlamlı olarak daha düşüktü. Nabız dalga hızı ile yaş (r: 0,204, p: 0,072), OPG (r: 0,219, p: 0,052), kalsiyumxfosfat çarpımı (r: 605, p <0,001) ve sistolik kan basıncı (r: 0,198 , p: 0,058) arasında pozitif korelasyon saptanırken, NDH ile RANKL (r: -0,261, p: 0,020) ve kreatinin klirensi (r: -0,220, p: 0,051) arasında negatif korelasyon tespit edildi. Lineer regresyon analizinde nabız dalga hızının bağımsız belirleyicisi olarak kalsiyum x fosfat çarpımı bulundu. Ancak kreatinin klirensi, RANKL osteoprotegerin ve sistolik kan basıncı NDH'nın bağımsız öngördürücüsü olarak tespit edilmedi.

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Received : 21.11.2014
Accepted : 03.03.2015

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SONUÇ: Çalışmamızda, böbrek nakilli hastalarda arteriyel sertliği gösteren nabız dalga hızı OPG ve RANKL ile değil fakat kalsiyum ve fosfor çarpımı ile bağımsız olarak ilişkili bulunmuştur.

ANAHTAR SÖZCÜKLER: Böbrek nakli, Osteoporoz, Osteoprotegerin, RANKL, Nabız dalga hızı

INTRODUCTION

Cardiovascular diseases (CVD) and mortality are increased even in the early stages of chronic kidney disease (CKD) and these increased risks are also found to be ongoing in end-stage renal disease (ESRD) patients who received dialysis therapy and underwent renal transplantation (Rtx) (1-2). Fifty to 60 percent of post-Rtx deaths were found to be associated with CVD, with an incidence of ischemic heart disease of approximately one per 100 persons a year at risk (3). Beside traditional risk factors including hypertension, diabetes, dyslipidemia, advanced age and left ventricular hypertrophy (LVH), novel risk factors such as endothelial dysfunction (ED), vascular calcification (VC), oxidative stress, arterial stiffness and inflammation are highly prevalent and seem to play a more important role in vascular disease in renal patients compared to healthy subjects (4-7). Osteoprotegerin (OPG) is a member of the tumour necrosis factor receptor superfamily and this molecule can be produced by osteoblasts and various cells of the vasculature. OPG was accepted as a cardiovascular risk factor in ESRD patients receiving hemodialysis (HD) (8) and undergoing Rtx (9). In this regard, increased serum OPG levels were found to be associated with increased coronary artery and aorta calcification in these populations indicating that OPG may be involved in the pathogenesis of VC (10). In addition, Hjelmesaeth et al. demonstrated that early post-transplant serum OPG levels might predict long-term patient survival and CV death in Rtx patients (9).

OPG, which is secreted by osteoblastic cells, acts as a decoy receptor binding receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL) and this interaction competitively inhibits binding of RANKL to its receptor RANK on osteoblasts. This results in inhibition of osteoclastic differentiation and in consequence inhibits bone resorption (11). Imbalances in the RANKL/OPG ratio or RANK signaling underlie the pathology of many skeletal disorders with excessive bone loss, excessive bone formation, or diseases with disordered bone remodeling (12). Osteoporosis and vascular calcification are commonly seen together in CKD patients. Hence, it has been speculated that OPG may represent a link between osteoporosis and arterial calcification (13) in CKD population.

Impaired aorta stiffening is commonly seen in CKD and Rtx patients (14,15). Measuring the pulse-wave velocity (PWV) is accepted as a reliable means of determining the aortic stiffness (16). Despite the beneficial effects of Rtx on CV risk, the exact role of renal transplantation in terms of aortic stiffness, blood pressure and osteoporosis is still unclear. To date, the data in the literature are limited regarding the relationship among the OPG/RANKL system, osteoporosis and CV events in Rtx patients. Hence, we sought to investigate the relation among OPG, RANKL, osteoporosis and endothelial dysfunction in Rtx patients with a well-functioning kidney.

MATERIALS and METHODS

Study Population

The study protocol was approved by the Medical Ethics Committee of Erciyes University Faculty of Medicine, Kayseri, Turkey (Ethics Committee Number: 2013/756). Written informed consent was obtained from all of the subjects included in the study.

This cross-sectional study included 80 adult renal transplantation recipients. We recorded levels of serum creatinine and hemoglobin, amount of proteinuria, serum albumin concentration, levels of intact parathormone (iPTH), body weight and height at the last follow-up. We also noted demographic data, immunosuppressive regimen, allograft source, and duration after transplantation. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Measurement of femoral neck mineral density was obtained by dual-energy X-ray absorptiometry (DEXA).

According to the World Health Organization (WHO) guidelines, osteoporosis is defined by a T score below -2.5, osteopenia by a score between -1.0 and -2.5, and normal by a score above -1.0. The risk of fracture is estimated to increase 1.5- to 3.0-fold for each standard deviation decrease in T score. It is important to note that T scores are valid only in older individuals. Z scores (i.e., the number of standard deviations from age-matched controls) are used in premenopausal women and men younger than 50 years of age, with a Z score below -2.0 indicating low bone mineral densitometry (BMD).

Serum osteoprotegerin was measured by a commercially available kit (Ray Biotech, Inc.), using the Ray Bio, Human Osteoprotegerin ELISA method. The intra-assay coefficient of variation was 10 % and the interassay variation was 12 %; average % recovery serum type sample was 99.85% as provided by the manufacturer. Serum RANKL (total) was measured by a commercially available kit (BioVendor-research and Diagnostic Products) using the BioVendor Human RANKL ELISA method. The intra-assay coefficient of variation was 7.25 % and the inter-assay variation was 11.21 %, as provided by the manufacturer.

Pulse Wave Velocity

Vascular studies were performed in a quiet, temperature-controlled room with subjects resting in a supine position. Systolic and diastolic blood pressures were measured in duplicate using a semi-automated, noninvasive oscillometric sphygmomanometer, following a 10-min rest period. Pulse-wave analysis measured in the carotid and femoral arteries using a pulse wave velocity (PWV) machine (Micro Medical Pulse Trace, Rochester, UK) in accordance with the manufacturer's recommendations. Briefly, the transducers were positioned over the carotid and femoral arteries, always on the right side of the body. PWV was automatically calculated by measuring the time for the pulse wave to travel between the carotid and femoral arteries. All measurements were performed over 15 heartbeats by a single operator blinded to the patient's grouping.

Ambulatory Blood Pressure Measurements

The 24-hour blood pressure monitoring was performed using a Del Mar Medical Pressurometer Model P6 (Del Mar Reynolds, Irvine, Calif., USA) and the results were assessed using the manufacturer's computer software. Ambulatory measurements were conducted once every 15 min from 7 a.m. until 11 p.m., and once every 30 min from 11 p.m. until 7 a.m. Evaluation was performed taking the mean values of day and night blood pressures into account. Hypertension was considered to be present if the systolic pressure was >140 mm Hg and/or diastolic pressure was >90 mm Hg, or if the individual was taking antihypertensive medication.

Statistical Analysis

All statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, Ill., USA). Continuous variables were tested for normal distribution by the Kolmogorov-Smirnov test. Continuous variables with normal distribution are presented as mean ± standard deviation (SD). The median value was used where normal distribution was absent. Statistical analysis for the parametric variables was performed using the Student's t-test between two groups. The Mann-Whitney U test was used to compare nonparametric variables between two groups. The correlation analysis was evaluated by Pearson's correlation test. Qualitative variables were given as percentages and the relationship between categorical variables was investigated using the chi-square test. Spearman correlation coefficients were calculated to examine the degree of association between variables. Multiple logistic regression analyses were performed to identify the risk factors for having osteoporosis/osteopenia in patients with renal transplantation. Odds ratios were calculated with 95% confidence intervals (CI). Significant variables at the 0.10 level were considered in the multiple model, and backward elimination was applied using the Wald statistic for pulse wave velocity. A p value <0.05 was considered as significant, and the confidence interval was set to 95%.

In logistic regression analysis, variables for which the unadjusted univariate p value was <0.10 in linear regression analysis were included. We reduced the model by using backward elimination multivariate linear regression analysis and compared remaining risk markers using likelihood ratio tests.

RESULTS

The mean age of the 80 patients was 40.6 ± 11.1 (range, 20-69) years; 47 (58.5%) of the 80 patients were male. The etiology of end-stage renal disease was diabetes mellitus in 11, hypertension in 15, glomerulonephritis in 6, polycystic kidney disease in 5, other causes in 10, and unknown in 33 patients. Preemptive transplantation was performed in only 8 patients whereas other 72 patients were on dialysis (46 patients in hemodialysis, 25 patients in peritoneal dialysis, and 1 patient received peritoneal dialysis and hemodialysis). Type of donor was living in 60 (75%) and cadaveric in 20 (25%) patients.

Table I shows comparison of the demographic, biochemical, and clinical parameters among the patient groups. There was a significant difference between two groups in terms of only body mass index (p:0.04). Body mass index was significantly lower in osteopenic/osteoporotic group compared to normal group. On the other hand, there were no significant differences among these groups with regard to other demographic, clinical, and biochemical parameters ($p > 0.05$).

Pulse wave velocity correlated with age (r: 0.204, p: 0.072), osteoprotegerin (r: 0.219, p: 0.052), calcium x phosphate product (r:0.605, p: <0.001), and systolic blood pressure (r: 0.198, p: 0.058). It was inversely correlated with RANKL (r: -0.261, p: 0.020) and creatinine clearance (r: -0.220, p: 0.051) (Figure 1). However, it did not correlate with body mass index, post-transplant duration, diastolic blood pressure, all serum lipids, lumbar total T and Z scores, osteoprotegerin/RANKL ratio, amount of proteinuria, serum iPTH, hs-CRP, hemoglobin and alkaline phosphatase ($p > 0.05$).

The independence of multiple correlations was analyzed with multivariate linear regression analyses. The original model included osteoprotegerin, RANKL, systolic blood pressure, creatinine clearance, and calcium x phosphate product. In all subjects, pulse wave velocity was independently predicted by calcium x phosphate product but not by creatinine clearance, RANKL, osteoprotegerin and systolic blood pressure (Table II).

In multiple analysis, each unit of increase in BMI led to a 0.87 decrease in the risk of osteoporosis/osteopenia (95% CI, 0.76-0.99) in univariate logistic regression analysis and 0.86 fold decrease (95% CI, 0.76-0.99) in multiple logistic regression analysis (Table III).

Table I: Comparison of demographic, biochemical, and clinical parameters between the patient groups.

	Normal group n=24	Osteoporotic/Osteopenia group n=56	p value
Age (year)	41.6±8.1	39.3±11.8	0.33
Gender			0.20
Male (no)	10	34	
Female (no)	14	22	
Type of donor			0.77
Living (no)	10	42	
Cadaveric (no)	14	14	
Body mass index (kg/m^2)	26.6±4.5	24.2±4.1	0.04
Post-transplant duration (months)	60.0 (4-168)	35.5(2-252)	0.41
Pulse wave velocity (m/sec)	7.8±1.1	7.3±1.3	0.13
Amount of proteinuria (mg/day)	0.17 (0.02-2.47)	0.20 (0.03-11.7)	0.55
Intact parathormone (pg/mL)	70 (28-354)	57.1 (14.9-1519)	0.41
hs-CRP (mg/dL)	3.45 (3.19-40)	3.45(3-108)	0.90
Serum alkaline phosphatase	81(41-339)	80.0 (45-195)	0.82
Serum glucose (mg/dL)	95.5 (66-182)	94 (71-222)	0.84
Serum calcium (mg/dL)	9.9±0.9	9.3±0.6	0.32
Serum phosphate (mg/dL)	2.86±0.6	3.01±0.8	0.41
Calcium x phosphate product (mg^2/dL^2)	26.09±5.7	27.92±8.6	0.35
Total cholesterol (mg/dL)	186±44.3	188±55.4	0.87
Triglyceride (mg/dL)	176.5 (80-243)	151 (35-416)	0.82
Low-density lipoprotein (mg/dL)	108±31.9	112±37.6	0.85
High-density lipoprotein (mg/dL)	43±12.1	44±13.5	0.61
Serum albumin (g/dL)	3.92±0.2	4.00±0.4	0.46
Serum uric acid (mg/dL)	5.43±1.4	6.25±1.8	0.06
Hemoglobin (g/dL)	13.3±1.8	13.8±1.8	0.22
eGFR (mL/min/1.73 m^2)	74.04±30.0	75.95±24.5	0.73
Osteoprotogerin (pg/ml)	178.7 (55.1-33.4)	145.5 (16.4-570.1)	0.82
RANKL(pmol/L)	5.1 (1.4-46.8)	4.5 (1.3-55)	0.90
Use of			
Steroid (%)	23 (95.8)	52 (92.9)	0.526
Cyclosporine-A (%)	1 (4.2)	8 (14.3)	0.180
Tacrolimus (%)	20 (83.3)	45 (80.4)	0.511
Mycophenolate (%)	21 (87.5)	47 (83.9)	0.782
Azathioprine (%)	1 (4.2)	4 (7.1)	0.526
mTOR inhibitors	3 (12.5)	2 (3.6)	0.156

hs-CRP: High-sensitive C-reactive protein, **RANKL:** Receptor activator of nuclear factor kappa-β ligand, **mTOR:** Mammalian target of rapamycin.

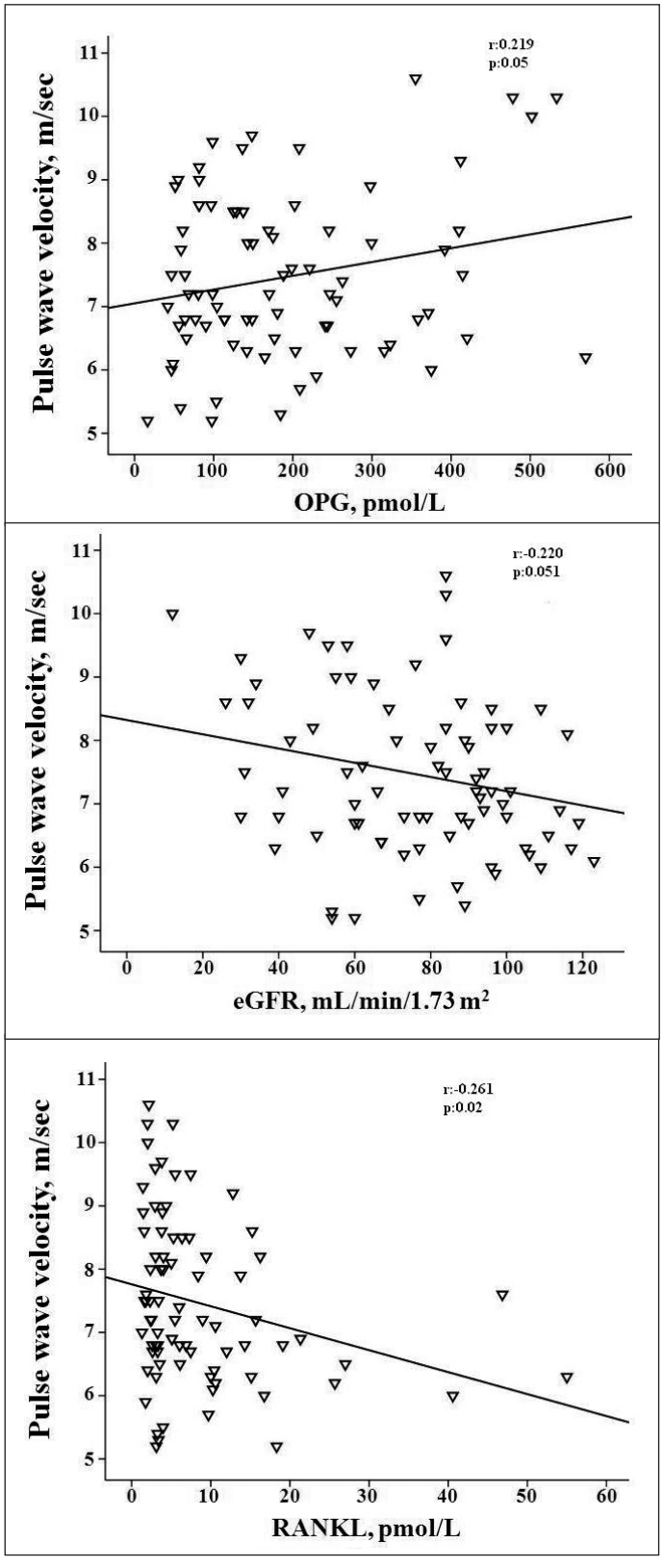

Figure 1: *Correlation of variables with PWV in Rtx patients.*

Table II: Multiple regression analysis backward method for pulse wave velocity.

	Pulse wave velocity		
	β	Standard error	p value
Systolic blood pressure	0.006	0.004	0.199
Creatinine clearance	-0.006	0.005	0.203
Calcium x phosphate product	0.594	0.015	<0.001
Osteoprotegerin	0.166	0.002	0.061

The original model included age, osteoprotegerin, RANKL, systolic blood pressure, creatinine clearance, and calcium x phosphate product. Adjusted $r^2 = 0.392$.

DISCUSSION

There were four main findings in the present study. First, BMI was found to be significantly lower in osteopenic/osteoporotic group when compared with non-osteopenic/osteoporotic Rtx patients. Second, PWV measurements were found to be positively correlated with age, systolic blood pressure, serum osteoprotegerin levels, and calcium x phosphate product and negatively correlated with creatinine clearance and serum RANKL levels. Third, among the variables mentioned above only calcium x phosphate product was found to be the main predictor of PWV in Rtx patients. Lastly, each unit of increase in BMI led to a 0.86 decrease in the risk of osteoporosis/osteopenia in univariate logistic regression analysis and a 0.86 fold decrease in multiple logistic regression analysis. To our knowledge, this is the first study in the literature to evaluate the role of serum OPG and RANKL on arterial stiffness in Rtx patients with osteopenia or osteoporosis.

Cardiovascular diseases are the most common cause of death with graft function after transplant and accounts for 30 percent of graft loss from death overall, with the highest rates early after Rtx (17). Transplant recipients have a lower risk of fatal and non-fatal cardiovascular events compared with waiting-list patients on dialysis (18,19). However, these patients have a much higher risk compared with the general population (20). Despite the improvement of kidney functions of this population after Rtx, estimated glomerular filtration rate places them in one of the stages of CKD. Renal transplant patients should therefore be considered a subset of CKD patients. Novel risk factors such as endothelial dysfunction, chronic kidney diseases-mineral bone disorders abnormalities including hyperphosphatemia, hyperparathyroidism and vascular-valvular calcification, increased oxidative stress, chronic low-grade ongoing inflammation and impaired aortic stiffness are highly prevalent and seem to play a more important role for vascular disease commonly seen in CKD and Rtx patients compared to healthy subjects (4-7, 21). Renal transplantation reduces CV risk

Table III: Univariate and multiple logistic regression analyses in presence of osteoporosis/osteopenia in patients with renal transplantation.

Variables	Univariate OR (95% CI)	p	Multiple OR (95%CI)	p
Age (years)	0.98 (0.94-1.03)	0.395	-	-
Gender				
Female	1.00	0.134	-	-
Male	2.11 (0.80-5.61)		-	-
BMI (kg/m^2)	0.87 (0.76-0.99)	0.046	0.87 (0.76-0.99)	**0.046**
Post-transplant duration (months)	1.00 (0.99-1.01)	0.786	-	-
Pulse wave velocity (m/sec)	0.75 (0.51-1.10)	0.137	-	-
Amount of proteinuria (mg/day)	1.15 (0.68-1.94)	0.609	-	-
Intact parathormone (pg/mL)	1.00 (0.99-1.01)	0.857	-	-
hs-CRP (mg/dL)	1.02 (0.98-1.06)	0.414	-	-
Alkaline phosphatase (mg/dL)	0.99 (0.98-1.00)	0.129	-	-
Serum glucose (mg/dL)	1.00 (0.98-1.02)	0.959	-	-
Calcium x phosphate product (mg^2/dL^2)	1.03 (0.99-1.01)	0.341	-	-
Triglyceride (mg/dL)	1.00 (0.99-1.01)	0.811	-	-
Low-density lipoprotein (mg/dL)	1.00 (0.99-1.01)	0.652	-	-
Serum albumin (g/dL)	1.64 (0.50-5.41)	0.413	-	-
Serum uric acid (mg/dL)	1.37 (0.98-1.90)	0.064	-	-
Hemoglobin (g/dL)	1.16 (0.89-1.51)	0.277	-	-
Creatinine clearance (mL/min)	1.00 (0.99-1.02)	0.764	-	-
Osteoprotogerin (pg/ml)	1.00 (0.99-1.01)	0.827	-	-
sRANKL (pmol/L)	1.02 (0.96-1.08)	0.517	-	-

OR: Odds ratio, **CI:** Confidence interval.

to some extent, but CV disease still remains the leading cause of death in renal transplant recipients (22). Beside the factors mentioned above, why the Rtx patients are more prone to worse CV outcomes and why these anticipated events remain after Rtx are still unclear. In a theory, the first step of this process is triggered by endothelial dysfunction and subsequently arterial stiffness, hovewer, in recent years several studies demonstrated that systemic persistent inflammation and VC particularly could be the main factors responsible for this increased CV risk in these patients regardless of the renal replacement therapy including Rtx (23). Arterial stiffness is increasingly recognized as an important potentially modifiable measure of subclinical vascular disease in general and CKD population (24). Data have emerged regarding the interaction between CKD mineral-bone disease and vascular disorders, that go beyond calcium and phosphate metabolism and secondary hyperparathyroidism (25). One of the important factors in this process is OPG and RANK-RANKL system (26). Animal studies have shown that OPG-deficient mice develop both severe osteoporosis and vascular calcification (27). OPG was found to affect bone formation and resorption and as well as the heart and kidneys (28). In addition, several studies have demonstrated that OPG is incorporated in atherosclerotic plaques (29,30). Conversely, elevated plasma levels of OPG are associated with increased mortality in high-risk diabetic patients (31), heart failure (32) and acute coronary syndrome (33) as well as in the general population (34). However, the exact mechanism for regulation of OPG in plasma and the presence of OPG in vascular and endothelial cells still remains unclear in both the CKD and RTx population.

In the present study, we preferred to determine PWV of Rtx patients as a direct measure of aortic stiffness. We demonstrated that advanced age, increased SBP, serum osteoprotegerin levels

and calcium x phosphate product are positively correlated with PWV implicating that as seen in CKD population, arterial stiffness might be partially mediated by OPG related cardiovascular risk in Rtx patients and negatively correlated with creatinine clearance and serum RANKL levels. In accordance with our results, Hotta et al. (35) demonstrated that PWV showed significant positive correlations with age, systolic blood pressure (BP), diastolic BP, and abdominal aortic calcification index in Rtx patients. Speer et al. (36) showed that serum OPG levels are strongly related to carotid-femoral PWV in ESRD patients receiving HD. Hence, we proposed that impairment of aortic stiffness might be one of the main CV risks responsible for increased CV events in the Rtx population.

Patients with CKD were found to be at high risk for the development of osteopenia and osteoporosis. The main risk factors for this undesirable process includes advanced age, postmenopausal age, glucocorticoid treatment or chronic heparin usage (37). Osteoporosis is present in both low and high-bone turnover states and bone loss begins much earlier in chronic HD patients than in general population (37). Baretto et al. (37) demonstrated that OPG/RANKL ratio was higher and OPG as well as OPG/RANKL ratio correlated negatively with trabecular bone volume in osteoporotic ESRD patients. It is possible that this process could be protective against bone loss by decreasing bone resorption. Doumouchtsis et al. (38) evaluated the clinical and biochemical correlations of bone BMD measurements in HD patients. The highest OPG levels were in the lowest T-score that means osteoporotic tertile and were higher than in osteopenic and normal tertiles. HD patients with low BMD of femoral neck demonstrated higher OPG levels than patients with normal BMD. This study showed that patients on dialysis for longer period had BMD below normal range (38).

In CKD patients there is a direct correlation between vascular calcification, cardiovascular risk and OPG level, just like in patients without chronic kidney disease. Chronically dialyzed children with calcification have higher OPG levels than those without this complication (39). Nitta et al. (40) concluded that the rapid progression of vascular calcification in HD patients was associated with higher OPG levels. In the present study, serum OPG and RANKL levels were found to be lower in osteopenic/osteoporotic Rtx patients compared to non- osteopenic/osteoporotic patients but the difference was not statistically significant. This might be secondary to relatively small number of our study population.

The present study had some limitations. First, this was a cross-sectional analysis of Rtx patients focusing on the relationship among serum OPG, RANKL and PWV. Second, the sample size was relatively small. This was not a prospective controlled study, so we cannot draw cause-and-effect relationships from our findings.

CONCLUSION

The discovery of OPG/RANK/RANKL system has highlighted our understanding in the role of regulation of bone remodeling and arterial stiffness in renal patients. However, recent studies in CKD and Rtx patients have produces many conflicting data regarding the role of OPG and RANKL in this era. In our study, serum calcium x phosphate product but not OPG and RANKL levels were found to be the main predictor of arterial stiffness in Rtx patients. Further studies are required to elucidate exact contribution of each OPG/RANK/RANKL molecule in the pathogenesis of vascular stiffness in RTx patients.

REFERENCES

1. Morris ST, McMurray JJ, Rodger RS, Farmer R, Jardine AG: Endothelial dysfunction in renal transplant recipients maintained on cyclosporine. Kidney Int 2000;57(3):1100-1106
2. Vanholder R, Massy Z, Argiles A, Spasovski G, Verbeke F, Lameire N: Chronic kidney disease as cause of cardiovascular morbidity and mortality. Nephrol Dial Transplant 2005;20(6):1048-1056
3. Ojo AO: Cardiovascular complications after renal transplantation and their prevention. Transplantation 2006;82(5):603-611
4. Parfrey PS, Foley RN, Harnett JD, Kent GM, Murray DC, Barre PE: Outcome and risk factors for left ventricular disorders in chronic uraemia. Nephrol Dial Transplant 1996;11(7):1277-1285
5. Cheung AK, Sarnak MJ, Yan G, Dwyer JT, Heyka RJ, Rocco MV, Teehan BP, Levey AS: Atherosclerotic cardiovascular disease risks in chronic hemodialysis patients. Kidney Int 2000;58(1):353-362
6. Muntner P, He J, Astor BC, Folsom AR, Coresh J: Traditional and nontraditional risk factors predict coronary heart disease in chronic kidney disease: Results from the atherosclerosis risk in communities study. J Am Soc Nephrol 2005;16(2):529-538
7. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM: The elephant in uremia: Oxidant stress as a unifying concept of cardiovascular disease in uremia. Kidney Int 2002;62(5):1524-1538
8. Morena M, Terrier N, Jaussent I, Leray-Moragues H, Chalabi L, Rivory JP, Maurice F, Delcourt C, Cristol JP, Canaud B, Dupuy AM: Plasma osteoprotegerin is associated with mortality in hemodialysis patients. J Am Soc Nephrol 2006;17(1):262-270
9. Hjelmesaeth J, Ueland T, Flyvbjerg A, Bollerslev J, Leivestad T, Jenssen T, Hansen TK, Thiel S, Sagedal S, Røislien J, Hartmann A: Early posttransplant serum osteoprotegerin levels predict long-term (8-year) patient survival and cardiovascular death in renal transplant patients. J Am Soc Nephrol 2006;17(6):1746-1754
10. Moe SM, Reslerova M, Ketteler M, O'neill K, Duan D, Koczman J, Westenfeld R, Jahnen-Dechent W, Chen NX: Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). Kidney Int 2005;67(6):2295-2304
11. Klejna K, Naumnik B, Gasowska K, Mysliwiec M: OPG/RANK/RANKL signaling system and its significance in nephrology. Folia Histochem Cytobiol 2009;47(2):199-206

12. Collin-Osdoby P: Regulation of vascular calcification by osteoclast regulatory factors RANKL and osteoprotegerin. Circ Res 2004;95(11):1046-1057
13. Hofbauer LC, Schoppet M: Osteoprotegerin: A link between osteoporosis and arterial calcification? Lancet 2001;358(9278):257-259
14. Minyukhina IE, Lipatov KS, Posokhov IN: Analysis of 24-hour pulse wave velocity in patients with renal transplantation. Int J Nephrol Renovasc Dis 2013;6:125-129
15. Chitalia N, Ismail T, Tooth L, Boa F, Hampson G, Goldsmith D, Kaski JC, Banerjee D: Impact of vitamin D supplementation on arterial vasomotion, stiffness and endothelial biomarkers in chronic kidney disease patients. PLoS One 2014;9(3):e91363
16. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, Target R, Levy BI: Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension 1995;26(3):485-490
17. Boddana P, Caskey F, Casula A, Ansell D: UK Renal Registry 11th Annual Report: Chapter 14 UK Renal Registry and international comparisons. Nephron Clin Pract 2009;111 Suppl 1:c269-276
18. Lentine KL, Brennan DC, Schnitzler MA: Incidence and predictors of myocardial infarction after kidney transplantation. J Am Soc Nephrol 2005;16(2):496-506
19. Meier-Kriesche HU, Schold JD, Srinivas TR, Reed A, Kaplan B: Kidney transplantation halts cardiovascular disease progression in patients with end-stage renal disease. Am J Transplant 2004;4(10):1662-1668
20. Jardine AG, Gaston RS, Fellstrom BC, Holdaas H: Prevention of cardiovascular disease in adult recipients of kidney transplants. Lancet 2011;378(9800):1419-1427
21. Turkmen K, Erdur FM, Guney I, Ozbiner H, Toker A, Gaipov A, Ozbek O, Yeksan M, Tonbul HZ, Turk S: Relationship between Plasma Pentraxin-3, Neutrophil-to-Lymphocyte Ratio, and Atherosclerosis in renal transplant patients. Cardiorenal Med 2012;2(4):298-307
22. Pilmore H, Dent H, Chang S, McDonald SP, Chadban SJ: Reduction in cardiovascular death after kidney transplantation. Transplantation 2010;89(7):851-857
23. Stenvinkel P, Carrero JJ, Axelsson J, Lindholm B, Heimburger O, Massy Z: Emerging biomarkers for evaluating cardiovascular risk in the chronic kidney disease patient: How do new pieces fit into the uremic puzzle? Clin J Am Soc Nephrol 2008;3(2):505-521
24. Vlachopoulos C, Aznaouridis K, Stefanadis C: Prediction of cardiovascular events and all-cause mortality with arterial stiffness: A systematic review and meta-analysis. J Am Coll Cardiol 2010;55(13):1318-1327
25. Demer L, Tintut Y: The bone-vascular axis in chronic kidney disease. Curr Opin Nephrol Hypertens 2010;19(4):349-353
26. Hruska KA, Choi ET, Memon I, Davis TK, Mathew S: Cardiovascular risk in chronic kidney disease (CKD): The CKD-mineral bone disorder (CKD-MBD). Pediatr Nephrol 2010;25(4):769-778
27. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS: Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998;12(9):1260-1268
28. Venuraju SM, Yerramasu A, Corder R, Lahiri A: Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. J Am Coll Cardiol 2010;55(19):2049-2061
29. Golledge J, McCann M, Mangan S, Lam A, Karan M: Osteoprotegerin and osteopontin are expressed at high concentrations within symptomatic carotid atherosclerosis. Stroke 2004;35(7):1636-1641
30. Sandberg WJ, Yndestad A, Øie E, Smith C, Ueland T, Ovchinnikova O, Robertson AK, Müller F, Semb AG, Scholz H, Andreassen AK, Gullestad L, Damås JK, Frøland SS, Hansson GK, Halvorsen B, Aukrust P: Enhanced T-cell expression of RANK ligand in acute coronary syndrome: Possible role in plaque destabilization. Arterioscler Thromb Vasc Biol 2006;26(4):857-863
31. Jorsal A, Tarnow L, Flyvbjerg A, Parving HH, Rossing P, Rasmussen LM: Plasma osteoprotegerin levels predict cardiovascular and all-cause mortality and deterioration of kidney function in type 1 diabetic patients with nephropathy. Diabetologia 2008;51(11):2100-2107
32. Ueland T, Jemtland R, Godang K, Kjekshus J, Hognestad A, Omland T, Squire IB, Gullestad L, Bollerslev J, Dickstein K, Aukrust P: Prognostic value of osteoprotegerin in heart failure after acute myocardial infarction. J Am Coll Cardiol 2004;44(10):1970-1976
33. Omland T, Ueland T, Jansson AM, Persson A, Karlsson T, Smith C, Herlitz J, Aukrust P, Hartford M, Caidahl K: Circulating osteoprotegerin levels and long-term prognosis in patients with acute coronary syndromes. J Am Coll Cardiol 2008;51(6):627-633
34. Anand DV, Lahiri A, Lim E, Hopkins D, Corder R: The relationship between plasma osteoprotegerin levels and coronary artery calcification in uncomplicated type 2 diabetic subjects. J Am Coll Cardiol 2006;47(9):1850-1857
35. Hotta K, Harada H, Sasaki H, Iwami D, Fukuzawa N, Morita K, Seki T, Togashi M, Nonomura K: Successful kidney transplantation ameliorates arterial stiffness in end-stage renal disease patients. Transplant Proc 2012;44(3):684-686
36. Speer G, Fekete BC, El Hadj Othmane T, Szabó T, Egresits J, Fodor E, Kiss I, Logan AG, Nemcsik J, Szabó A, Németh ZK, Szathmári M, Tislér A: Serum osteoprotegerin level, carotid-femoral pulse wave velocity and cardiovascular survival in haemodialysis patients. Nephrol Dial Transplant 2008;23(10):3256-3262
37. Barreto FC, Barreto DV, Moyses RM, Neves CL, Jorgetti V, Draibe SA, Canziani ME, Carvalho AB: Osteoporosis in hemodialysis patients revisited by bone histomorphometry: A new insight into an old problem. Kidney Int 2006;69(10):1852-1857
38. Doumouchtsis KK, Kostakis AI, Doumouchtsis SK, Tziamalis MP, Stathakis CP, Diamanti-Kandarakis E, Dimitroulis D, Perrea DN: Associations between osteoprotegerin and femoral neck BMD in hemodialysis patients. J Bone Miner Metab 2008;26(1):66-72
39. Shroff RC, Shah V, Hiorns MP, Schoppet M, Hofbauer LC, Hawa G, Schurgers LJ, Singhal A, Merryweather I, Brogan P, Shanahan C, Deanfield J, Rees L: The circulating calcification inhibitors, fetuin-A and osteoprotegerin, but not matrix Gla protein, are associated with vascular stiffness and calcification in children on dialysis. Nephrol Dial Transplant 2008;23(10):3263-3271
40. Nitta K, Akiba T, Uchida K, Otsubo S, Takei T, Yumura W, Kabaya T, Nihei H: Serum osteoprotegerin levels and the extent of vascular calcification in haemodialysis patients. Nephrol Dial Transplant 2004;19(7):1886-1889